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1.932
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June, 1952



AIC-337

DETERMINATION OF PECTIC SUBSTANCES AND PECTIC ENZYMES IN
CITRUS AND TOMATO PRODUCTS

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This report is concerned with pectic substances in tomatoes and citrus fruits. Methods of extraction, isolation, and analysis, based on the solubility characteristics of the pectic substances, are presented, and procedures for the measurement of activities of various pectic enzymes in these materials are also included. Discussions of the various procedures, with examples, are included to aid the reader in his choice of the most suitable procedure and in his interpretation of results.

Bureau of Agricultural and Industrial Chemistry.
Agricultural Research Administration
UNITED STATES DEPARTMENT OF AGRICULTURE

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DETERMINATION OF PECTIC SUBSTANCES AND PECTIC ENZYMES IN CITRUS AND TOMATO PRODUCTS

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The effects of pectic substances and their enzymatic alteration on the quality of food products are becoming more and more apparent to the food technologist. It has become desirable in industries concerned with certain foods to investigate the pectic substances present and the nature of the changes they may undergo during processing or storage.

There are available numerous methods for the determination of pectic substances. These methods, however, are widely scattered in the literature and are not always readily located. In addition, most published methods have certain individual advantages and disadvantages that require employment of considerable knowledge of types of pectic substances and their effects in food products in the selection of the most suitable method for a particular case and in interpreting the results.

The purpose here is to point out variations in the pectic substances of citrus and tomato products and the type of information that may (or may not) be obtained by the application of different analytical methods. A bibliography of some of the most important analytical methods is included.

Nearly all of the satisfactory methods for the determination of pectic substances tend to be unwieldy and time consuming, and probably not adaptable to industrial application. Therefore this report does not present a detailed description of each procedure; instead it presents the nature of the methods and the type of information needed so that the food analyst, by judicious choice and combination of procedures, together with a considered interpretation of results, can obtain useful information with minimum effort. A particularly important point, overlooked in some procedures, is the fact that water does not extract the low-ester or enzyme-demethylated pectic substances from food material.

Because of their relationship to changes in pectic substances, descriptions of the actions and methods of estimation of the pectic enzymes are included.

Pectic Substances

The nomenclature adopted by the American Chemical Society (8) defines four types of pectic substances: protopectin, pectinic acids, pectin, and pectic acid. For definitions of these terms, one should see the literature cited. From the standpoint of analysis and influence in food products, these pectic substances can be placed in three arbitrary groups, according to their solubility characteristics.^{1/} These groups with some of their important characteristics are as follows:

^{1/} The pectic substances included in either of these classifications are by definition of high-molecular-weight and colloidal, as contrasted to low-molecular-weight degraded pectic substances, which do not contribute to consistency or viscosity.

A "Water-Soluble" Fraction: Includes pectin and those colloidal pectinic acids of sufficiently high methyl ester content to be water-soluble. They are the high-molecular-weight, soluble pectic substances, located largely in the liquid phase of a food product, which contribute to consistency and serum viscosity. In addition, "water-soluble" pectic substances act as colloid stabilizers and play an important role in preventing the settling or flocculation of the dispersed solid phase in products such as tomato and citrus juices.

An "Oxalate-Soluble" Fraction: Includes pectic acid and those colloidal pectinic acids of sufficiently low methyl ester content to be insoluble in water (but soluble in oxalates, polymetaphosphates such as sodium hexametaphosphate, and other calcium-sequestering agents), and capable of forming gels or precipitates with polyvalent metal ions. These pectic substances may occur naturally in small amounts or may be formed in larger amounts through the action of the enzyme, pectin-methylesterase (pectase, pectinesterase), on water-soluble pectic substances.

The effect of low-ester pectic substances is dependent on their concentration and on the pH and metal ion content of the medium. In relatively high concentrations, they may contribute to high consistency by virtue of their tendency to form gel structures with polyvalent metal ions. This effect may be desirable in the case of tomato pastes but is definitely undesirable in the case of citrus concentrates. At lower concentrations the formation of salts with polyvalent metal ions may cause the formation of unsightly flocculent precipitates rather than a gel.

An "Acid-Soluble" Fraction (Protopectin): An ill-defined water-insoluble substance in plant tissue which on restricted hydrolysis (viz., warm, dilute acid) yields pectinic acids. The importance of protopectin in foods is the likelihood that over extended storage periods slow hydrolysis of this material may cause an increase in the pectin or pectinic acid content of the product. Protopectin itself may have some effect in food products as a large insoluble water-binder, and in this manner might make a contribution to consistency. Protopectin of the tomato fruit presumably becomes soluble upon heating for several hours in 0.05N HCl at 80-90°C.

Method of Preparing Samples and Extracting Pectic Substances.^{2/} In general, 25 ml. of the sample (or sufficient sample to contain 25-100 mg. of pectic substances) is taken, and two volumes of 95 percent ethanol are added plus one or two drops of conc. HCl to increase the solubility of inorganic salts. This preparation is brought to a boil to inactivate any pectic enzymes and rapidly cooled. Samples can be stored for some time in this ethanol mixture.

Prior to extraction of the pectic substances, the supernatant is filtered from the prepared sample on sharkskin paper^{3/} and the sample is washed on the paper several

^{2/} In part, the scheme of extractions here presented is taken from Z. I. Kertesz and R. J. McColloch, New York State Agr. Exp. Sta. Bull. 745 (1950).

^{3/} Sharkskin paper is employed for all filtrations because of its speed and hardness.

times with a mixture of two parts 95 percent ethanol to one part water plus sufficient conc. HCl to make the mixture 0.05N with respect to HCl. The washing removes additional interfering materials.

Preparation of "Water-Soluble" Extract: The pulp is transferred to a 125-ml. Erlenmeyer flask with 50 ml. of distilled water and extracted by standing two hours at 30°C. with frequent mixing. The supernatant is filtered off and saved and the water extraction of the pulp repeated. The filtrates from the first and second extractions are combined and this mixture contains "water-soluble" pectic substances.

Preparation of "Oxalate-Soluble" Extract: The pulp from the last water extraction is transferred to the Erlenmeyer flask with 50 ml. of 0.2 percent ammonium oxalate solution. Two extractions for two hours each at 30°C. are made in the manner of the water extractions and the combined filtrates constitute "oxalate-soluble" pectic substances.

Preparation of "Acid-Soluble" Pectic Substances: The pulp from the last oxalate extraction is transferred to the Erlenmeyer with 50 ml. of 0.05N HCl solution.^{4/} Two extractions for two hours each are employed in the previously described manner, except that a temperature of 80-90°C. is maintained during the extraction. The resulting combined filtrates contain "acid-soluble" pectic substances.

Preparation of the Extracts for Analysis: The pectic substances in each of the three extracts mentioned in the paragraphs above are precipitated in 500-ml. Erlenmeyer flasks by the addition of two volumes of 95 percent ethanol and sufficient conc. HCl to make the mixture 0.05N. These preparations can be stoppered and stored for later analysis. When analyses are to be made, the supernatant ethanol-water mixture is filtered off and the precipitated pectic substances are washed at least three times on the paper with a mixture of two parts of 95 percent ethanol to one part water, this mixture containing 0.05N HCl. The precipitate on the paper may then be employed for any one of the various types of analyses as described later.

Discussion of the Extractions: The order of the extractions from a mild solvent to the stronger hot acid is important. Hot acid for example might hydrolyze "water-soluble" fractions to low-molecular-weight polyuronides, which would not be determined by the method later employed or, if determined, would give a false picture of the pectic make-up of the original material. It is also important to determine all of the three types of pectic substances unless prior information gained by this type of analysis makes it possible to interpret changes correctly in only one or two of the fractions.

To illustrate, consider a sample of citrus concentrate containing active pectin-methylesterase. A complete analysis of such a product over a storage period might indicate that "oxalate-soluble", low-ester pectinic acids increased in amount at

^{4/} Preliminary evidence makes it appear likely that in the case of citrus materials, a stronger acid hydrolysis should be employed in order to recover all the protopectin.

the expense of "water-soluble", high-ester pectinic acids which were being enzymatically demethylated. At the same time there might be a slow conversion of "acid-soluble" protopectin to pectin, reducing the rate at which "water-soluble" pectic substances disappeared with an increase in "oxalate-soluble" pectic substances.

An analysis of only "water-soluble" materials of the same sample might falsely indicate (a) a loss of pectic substances (conversion of "water-soluble" to "oxalate-soluble"), (b) no change if rate of hydrolysis of "protopectin" to "water-soluble" pectinic acids equaled the rate of demethylation, or (c) an increase in pectic substances if rate of hydrolysis of "protopectin" exceeded rate of demethylation. Similarly, single determinations of only "oxalate-soluble" or "not acid-soluble" pectic substances may give misleading results.

To illustrate these statements, consider the following table of data obtained during a study of cloud loss and pectic changes in orange concentrate at elevated temperatures.

Pectin Data from Citrus Storage Studies

Description of concentrate sample	Sample No.	"Water- soluble"	"Oxalate- soluble"	"Acid- soluble"	Total	Cloud readings ^{1/}
		<u>mg./25-gm. sample</u>				
Held at -95°F. 12 hrs., thawed	(1)	11	12	61	84	--
Stored 3 months at 0°F.	(2)	10	13	61	84	410
Thawed 1 day 50°F. stored 3 mo. 0°F.	(3)	2	25	56	83	169
Thawed 2 days 50°F. stored 3 mo. 0°F.	(4)	5	23	46	74	133
Thawed 3 days 50°F. stored 3 mo. 0°F.	(5)	0	13	40	53	68

^{1/} Arbitrary colorimeter readings.

The data show that cloud loss was associated with two types of pectic changes occurring in the samples. The initial large loss in cloud (Samples 2 and 3) was associated with the enzymatic conversion of "water-soluble" highly esterified pectinic acids to low-ester "oxalate-soluble" pectinic acids. Later losses in cloud (Samples 3,4,5) were due to a gradual decrease in total pectic substances. This destruction of pectic substances was traceable to the presence of a fungal-type pectin-polygalacturonase associated with the very high microbiological population of the concentrate samples.

The pectic changes occurring in frozen orange concentrate under other conditions may be more complex, and it can be seen that a complete analysis of pectic substances is necessary when it is desired to explain the mechanisms of the changes involved. In the example above, analysis of the "water-soluble" material alone would have falsely indicated that the initial large cloud loss was due to a change in the amount of pectic substance rather than to a change in the kind of pectic substance. Measurement of the total pectic substances would have given no explanation of the initial cloud loss.

When the mechanisms involved are well understood, however, single determinations may be suitable. For convenience in any "free-hand" modifications which may appear to be desirable, the following table of the solubility relationships of the three types of pectic substances may prove useful.

Solubility of Pectic Substances

Type of pectic substance	Solubility in		
	<u>Water</u>	<u>Oxalate</u>	<u>Hot acid</u>
"Water-soluble"	Yes	Yes	Yes, may be hydrolyzed
"Oxalate-soluble"	No (or slight)	Yes	No, except on exhaustive treatment
"Acid-soluble"	No (or slight)	No (or slight)	Yes

Methods of Determining Pectin Directly in the Ethanol Precipitate

After the various types of pectic substances have been extracted and partially purified, the actual quantitative analysis can be made by a large variety of methods. Each has its individual advantages and disadvantages, which will be pointed out. Actual choice should depend on equipment at hand, degree of accuracy desired, type of information wanted, and time available. The methods described below are direct determinations, which can be applied to the samples obtained by methods mentioned above under "Preparation of Extracts for Analysis", page 3). A more indirect method (calcium pectate) is described separately below on page 6.

Estimation of the Alcohol Precipitate: The precipitated pectic substances can be crudely estimated by centrifuging under standard conditions in a graduated centrifuge tube and estimating the volume of precipitate. Besides the usual errors to which this type of determination is subject, a considerable error occurs when the pectic substances vary in grade or molecular weight. Equal weights of pectic substances of different molecular weights will give different volumes of precipitate.

A more suitable method is to collect the ethanol precipitate in a weighed platinum dish, after which it can be dried and weighed. Additional accuracy can be achieved by ashing the dried precipitate and correcting the dry weight for ash content.

Polyuronide CO₂ Determination: There are a number of published methods for the determination of polyuronide material based upon the quantitative yield of CO₂ obtained when the polyuronide is heated with strong acid. In the method of Nanji (14), the CO₂ liberated is passed through barium hydroxide solution, forming barium carbonate, and the change in the barium hydroxide concentration is determined by titration. The method of Whistler, Martin, and Harris (17) requires 6 to 8 hours and the CO₂ liberated each hour is accurately determined by gravimetric weighings. From the resulting rate curve of CO₂ evolution, corrections can be applied for the "non-uronide" CO₂ liberated. McCready, Swenson, and MacLay (13) have reduced the time required in the CO₂ method by employing higher concentrations of acid and higher temperature, although there is perhaps a small sacrifice in accuracy.

Colorimetric Determination: A colorimetric method for the determination of uronic acids employing the development of color with carbazol has been elaborated by Dische (3). This method has not as yet been applied to the determination of pectic substances in food products; however, it shows sufficient promise to insure an early trial.

Discussion of Direct Methods: An inherent disadvantage in the methods outlined in preceding sections becomes apparent in considering the composition of the ethanol precipitate. Those pectic substances which fit our definitions and contribute to consistency, viscosity, and the stabilization of colloidal suspensions must be of high molecular weight and colloidal. Ethanol of 60-70 percent concentration (2 ethanol:1 water), however, also precipitates low-molecular-weight polyuronides which are not colloidal. These types of determinations, therefore, yield values of the total ethanol-insoluble pectic material irrespective of its ability to contribute to gelation, consistency, etc.

An additional disadvantage of the methods employing CO₂ determination is their lack of specificity in complex mixtures of biological materials. It is known that ascorbic acid, for example, yields CO₂ quantitatively and at the same rate as galacturonic acid and the polyuronides. Although ascorbic acid would largely be eliminated from the determination by the ethanol fractionation, there is no assurance that higher-molecular-weight alpha-keto and beta-hydroxy acids, which may readily yield CO₂ under the conditions of the methods, will not be present as impurities in the alcohol precipitate. There is less possibility that the substances will interfere in the Carré-Haynes calcium pectate method described later.

Determination of Pectic Substances as Calcium Pectate

The Carré-Haynes calcium pectate method (2) has for many years been a classical method for the determination of pectic substances. The following description is an adaptation of this method as employed in this Laboratory, by which a simultaneous determination of the pectic substances as well as their methoxyl content is obtained.

The ethanol precipitate of pectic material is washed through the filter paper into a 500-ml. Erlenmeyer flask with boiling water. With the precipitate from the oxalate extractions, it may be necessary to render the water slightly alkaline with a drop or two of 0.1N NaOH to facilitate solubilization. Preferably while the filtrate is still hot, a few drops of the Hinton (5) indicator^{5/} are added to the filtrate, which is then quickly titrated to pH 7.5 (indicator just turns purple). Exactly 10 ml. of 0.5N NaOH is added and the flask is stoppered and allowed to stand 30 minutes. A blank of distilled water (hot) is carried through in the identical manner. At the end of 30 minutes the analytical samples and the water blank are back-titrated to the neutral point with standard 0.1N HCl. The difference between the blank titration and the titrations of the samples represents the base consumed in the saponification of the methyl ester groups. (One ml. of 0.1000N NaOH is equivalent to 3.1 mg. of methoxyl (MeO).)

Following the titration for methoxyl groups, 10 ml. of 1N acetic acid is added with stirring and the sample is allowed to stand 10 minutes. Then 10 ml. of 0.1M CaCl₂ and 10 ml. of 2.0M CaCl₂ are added in succession with vigorous stirring. The sample may then be brought to a quick boil for one minute or placed on a steam bath 15-30 minutes to aid consolidation of the calcium-pectate precipitate.

After settling, the precipitate is filtered on sharkskin paper and the calcium pectate washed thoroughly 2 or 3 times on the paper with hot distilled water to remove excess calcium ions. The precipitate is then washed off the paper into a small beaker (25-50 ml.)^{6/} and transferred quantitatively to a clean, dried, and weighed sintered glass (medium pore size) crucible, where it is again filtered and washed, with suction to facilitate the process. The precipitate is dried overnight in the crucible at 85°C. and weighed.

Crucibles are best cleaned after determinations by gently scraping out the gross deposits of calcium pectate and allowing a saturated solution of ammonium oxalate to filter through the crucible under gravity. This should be repeated at least twice; then the crucible is washed with distilled water and distilled water is run through the sinter under vacuum. Crucibles can then be rinsed with ethanol to facilitate drying and dried overnight at 85°C.

Discussion of Calcium Pectate Method: An advantage of the calcium pectate method in conjunction with the solubility fractionation of the pectic substances is that only those pectic substances which are of sufficiently high molecular weight to be colloidal form a calcium-pectate precipitate. Therefore the amounts of pectic

5/ Sodium salts of: Bromothymol Blue (0.4 percent) - 1 volume
Phenol Red (0.4 percent) - 3 volumes
Cresol Red (0.4 percent) - 1 volume
Distilled water - 1 volume

6/ At this point the precipitate could be dried in a platinum dish and weighed, and then ashed and re-weighed as an alternative gravimetric method.

substances thus determined can be interpreted significantly with respect to their effects on colloid stability, viscosity, and gel formation. Those pectic substances of low molecular weight (and galacturonic acid), which would not be expected to affect the above properties, do not give insoluble calcium pectates.

On the other hand this method is occasionally subject to errors caused by denatured and salted-out proteins that occur in the calcium-pectate precipitate. A much more serious disadvantage of the method is the time required. Including the three pairs of extractions, the calcium-pectate method will require at least two days and part of a third for completion. This fact alone has greatly limited the usefulness of the method except as a research tool.

Perhaps the most careful determination of pectic substances would combine separation of the various solubility fractions, followed by calcium-pectate formation to screen out the lower-molecular-weight polyuronides, and followed then by the accurate CO₂ method applied directly to the calcium pectates. This procedure would of course extend further the time required for the determinations.

It is apparent that no rapid satisfactory method for the complete analysis of pectic substances is as yet available. Studies of methods are now under way and it is hoped that more satisfactory procedures will soon be developed. Nevertheless by judicious choice and combinations of the methods, much useful information can be obtained.

Determination of Pectin-Methylesterase (Pectase, Pectinesterase)

Because of its importance in the tomato industry where the action of pectin-methylesterase initiates degradative changes in pectic substances and in the citrus industry where its action leads to gelation and/or cloud loss, a brief survey is here included of methods which can be employed to determine pectin-methylesterase quantitatively.

A number of methods have been described for the determination of pectin-methylesterase activity in biological materials (4, 7, 9, 10). In these methods, the rate of demethylation of the pectinic acids by the action of the enzyme at its optimum pH (7.0-7.5) is measured. This is accomplished by continuously titrating the liberated carboxyl groups with standard 0.1N NaOH, maintaining the pH at a constant value (7.5), and measuring the base consumed in a given interval of time. Pectin-methylesterase units (PMU) are expressed as mg. of methoxyl liberated in 30 minutes per ml. of enzyme preparation; that is:

$$\text{PMU} = \frac{(\text{ml. } 0.1000\text{N NaOH consumed}) (3.1) (30 \text{ minutes})}{(\text{ml. enzyme preparation}) (\text{actual minutes of the determination})} \cdot$$

The methods differ mainly in the preparation of the pectin substrate with respect to salts and in the units for expressing enzyme activity.

One of the most convenient substrates for this determination is a 0.5 percent solution of Pectinum (National Formulary) containing 0.1M sodium chloride. This substrate is usually prepared as needed from a 2 percent salt-free solution of Pectinum, which has better keeping qualities. As will be seen later, the proper salt concentration can be achieved either by adding salt directly to the pectin solution or in many cases (where enzyme extraction is employed), by adding a calculated amount of the salt extract of the enzyme directly to the 0.5 percent solution of Pectinum.

In the determination of pectin-methylesterase in tomato tissues, few difficulties are encountered because the activity present is relatively high. In the case of some citrus products, it is often difficult to obtain the amount of activity desired for rapid and accurate measurements, without employing excessive amounts of the material under investigation. A device which is sometimes helpful in the latter case is extraction of the enzyme from the tissues in which it is largely adsorbed. This can be accomplished by adding sufficient dry sodium chloride to the material to give a 10 percent solution and bringing the pH of the preparation to about 7.0 by addition of sodium hydroxide (12). After standing for about an hour, particularly if extraction is assisted by comminuting or grinding the tissues, most of the enzyme activity present will be obtained in the filtered extract. An advantage of this procedure is that the enzyme extract is usually relatively colorless, so that an indicator titration can be used rather than a pH meter. This enables the operator to run several determinations simultaneously. The most useful indicator for this titration is the same Hinton indicator (5) originally developed for determining the methyl-ester groups in pectin. This indicator has a sharp end-point at pH 7.0 to 7.5, which is also the optimum pH range for determining pectin-methylesterase activity.

An approximate adaptation of the method, which is at least suitable when it is necessary to determine relative amounts of the enzyme in a large number of samples, consists of making up reaction mixtures of enzyme extract with the pectin solution and Hinton indicator and counting the number of drops of base required in a given time to maintain a constant pH of 7.5 in each sample.

Determination of Pectolytic Enzymes

It is now clear that there are several types, or mixtures, of enzymes (11, 16) capable of reducing the molecular weight of pectin or pectic acid presumably through hydrolysis of the glycoside bonds. These enzymes, or mixtures, possess in common the ability to reduce the molecular weight and colloidal nature of some pectic substances and to destroy the viscosity of their solutions. On the other hand the enzymes appear to differ markedly in their mode of action and the extent of hydrolysis produced (11, 16). It is essential that these differences be considered in choosing a method for determining the enzyme activity present and in interpreting the results obtained.

Pectin polygalacturonase (PG) is a pectolytic enzyme of mold origin which is capable of hydrolyzing pectic acid to the monomer, galacturonic acid. During the course of action of this enzyme, the high-molecular-weight properties of the substrate, such as calcium and alcohol precipitability, are gradually reduced,

becoming vanishingly small when 50 to 80 percent of the total reducing-power increase has occurred. Pectic acid depolymerase (DP) is a newly described (10,11) pectolytic enzyme occurring in the tomato fruit. It does not appear to be capable of more than about 50 percent hydrolysis of pectic acid (judged by reducing-power increase), and its mode of action on pectic acid is such that calcium precipitability and ability to form a colloidal alcohol precipitate rapidly become vanishingly small when only 20 percent of the theoretical total increase in reducing power, based on polygalacturonase action, has occurred. For this reason an amount of DP which would appear almost insignificantly small on the basis of reducing-power-increase measurements can cause a complete loss of colloidal pectic substances in unheated tomato macerate in 1 to 10 minutes.

The various methods of measuring pectolytic enzyme action have been pointed out by Phaff and Joslyn (15). Also the work of Jansen and MacDonnell (6) has made it clear that a pectic acid substrate must be employed for such measurements. Among those methods in use for the measurement of pectolytic enzyme action are: change in viscosity with time, change in weight of calcium pectate precipitates, change in volume or character of 70 percent ethanol precipitates, change in optical rotation, and increase in reducing power. Probably the most suitable quantitative method is one based on measurement of the increase in reducing power accompanying the hydrolysis of the alpha-D-galacturonide bonds.

The most popular method of reducing-power measurement for this determination is the Willstätter-Schudel hypoiodate method (1). Jansen and MacDonnell (6) have described a modified Willstätter-Schudel method for the measurement of PG activity in a reaction mixture at pH 4.0. In the case of DP, its optimum pH of 4.5 should be employed. A somewhat simpler adaptation of the Willstätter-Schudel method in use at this Laboratory is as follows:

Use 2 ml. of the enzyme solution or extract in question in 50 ml. of 0.25 percent pectic acid^{7/} solution previously adjusted to pH 5.0. Sufficient NaCl should be added to the substrate (1 ml. sat. NaCl solution) to make it approximately 0.1M with respect to NaCl and the pH adjusted to 4.5 for DP measurements and 3.5 for PG measurements. The adjustment to pH 4.5 is usually accomplished automatically by addition of the salt solution and in the case of 50 percent NaCl-saturated extracts of tomato DP, the salt and pH adjustments are accomplished simply by addition of the 2 ml. of enzyme solution. The reaction mixture is incubated at 30°C. and 10-ml. samples are taken at zero time for reducing-power determinations, and at subsequent intervals ad lib., depending on the activity encountered.

The 10-ml. sample of reaction mixture is pipetted into a 125-ml. Erlenmeyer flask. Exactly 10 ml. of standard 0.1N iodine solution is added, followed by 15 ml. of 0.1N NaOH. The mixture is stoppered and allowed to stand 15 minutes at room temperature. Then 20 ml. of 0.1N HCl are added and the excess iodine titrated with standard 0.1N thiosulfate reagent, with starch indicator.

^{7/} If traces of polyvalent metal ions in the enzyme preparation cause precipitation of pectic acid, 0.1 percent solution can be employed.

Because of lack of pH and temperature control in the reducing-power determination, this method no doubt lacks precision. However, since the measurement of activity is based on the differences between successive reducing-power determinations, many of the errors will automatically cancel out and the overall comparative values found agree favorably with those found by somewhat more laborious procedures.

The fact that the rate of change of molecular weight with increase in reducing power may vary with different types of enzyme must be taken into account in the interpretation of results. From the standpoint of pectic substances in foods, it is not sufficient to estimate only the rate of glycoside-bond hydrolysis; one must also determine the relationship between this change and the more practically important change produced in the colloidal nature of the pectic substance. This has become evident in the tomato-processing industry where the amount of pectolytic activity found by reducing-power methods would not account for the observed rapid losses in pectic substances if the mode of the action measured were similar to PG. It has been necessary to show by calcium-pectate determinations that the rate of molecular-weight degradation per glycoside bond broken is much greater for the tomato DP than for the PG of molds.

One cannot be too cautious in interpreting results based only on methods which measure a property of high molecular weight such as change in viscosity, calcium-pectate formation, or alcohol precipitability. All of these properties disappear in the earlier stages of hydrolysis in the order: viscosity, calcium-pectate formation, and alcohol precipitability. The viscosity of pectin and pectic-acid solutions is notoriously sensitive to factors other than enzymatic hydrolysis. Long standing, mild heating, presence of ascorbic acid and/or peroxides are among some of the factors capable of causing marked losses in the viscosity of a pectin or pectic acid solution under some conditions. It is therefore never safe to take the change in viscosity of these solutions as a measure of enzyme activity unless the identity of enzyme action has been established or confirmed by one or more other types of measurement.

In summary, any investigation of a new pectolytic action should initially include more than one type of measurement to indicate the mode of action and prove that enzymatic hydrolysis is involved. There are at least two types of enzymatic breakdown of pectic acid known at the present time and it is likely that others also exist.

Conclusion

The purpose of this report has not been an attempt to describe in detail all methods of analysis of pectic substances and pectic enzymes, but rather to indicate the nature of these analyses in such a form as to suggest their suitability or shortcomings for particular applications. Probably the most important considerations herein contained are those relating to the solubility differences exhibited by the different types of pectic substances, the general usefulness and applicability of the Hinton indicator in the determination of pectin-methyl-esterase activity, and the precautions relating to the interpretation of information obtained by the different types of pectic substance and pectolytic enzyme analyses.

It should be pointed out again that in spite of its unfortunate complexity, the calcium pectate method is at present the one which most satisfactorily determines only those pectic substances that are colloidal and contribute to consistency and viscosity. It would be advisable in the use of any other type of determination to establish at least some relationship for the particular case between the analysis by the calcium-pectate method and the method in question. The same applies to determinations of pectolytic enzyme action.

The analyst must choose for himself the most suitable methods from the overall standpoint of time and convenience. If the information in this report assists in that choice, however, a useful purpose has been served.

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